CLINICAL PROFILE AND RISK FACTORS OF STEROID RESISTANT NEPHROTIC SYNDROME IN AN URBAN REFERRAL CENTRE

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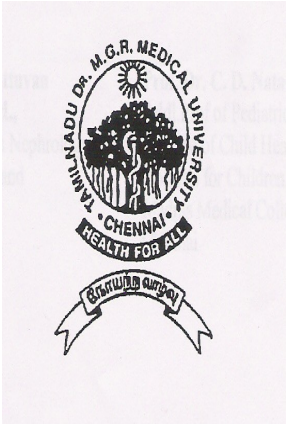

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CERTIFICATE

Certified that this dissertation entitled ***"CLINICAL PROFILE AND RISK FACTORS OF STEROID RESISTANT NEPHROTIC SYNDROME IN AN URBAN REFERRAL CENTRE"*** *is a bonafide work done by* ***Dr. R. ARIVAZHAGAN, M.D****., Postgraduate student of Pediatric Medicine, Institute of Child Health and Hospital for Children, Egmore, Chennai –8 attached to Madras Medical College, during the academic year 2005-2008.*

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CONTENTS

INTRODUCTION

The term nephrosis, or nephrotic syndrome, had its origin in the early 20th century and was introduced primarily to distinguish it from nephritis, a label used to denote a clinical state associated with hematuria, proteinuria, and cellular proliferation of the glomerulus. It describes a clinical condition of edema and proteinuria in which the renal histology (light microscopy) demonstrates fatty degeneration of the tubules associated with normal appearing glomeruli. Briefly, the name was modified to lipoid nephrosis after the routine finding of lipid droplets in the urine of affected patients.

Nephrotic syndrome is characterized by heavy proteinuria (>3.5 gm/day in adults or 40mg/m^2/hr in children), hypoalbuminemia (<2.5 gm/dl), edema and hyperlipidemia [(1).] It is primarily a pediatric disorder and is 15 times more common in children than adults.

Etiology

Most children (90%) with Nephrotic syndrome have a form of the primary Nephrotic syndrome. 10% of children with Nephrotic syndrome have secondary Nephrotic syndrome [(2)]

A. Causes of primary Nephrotic syndrome includes

1. Minimal change nephrotic syndrome (MCN) (85%)
2. Mesangial proliferation (5%)
3. Focal segmental glomerulo sclerosis (FSGS) (10%)
4. Membranous nephropathy (MGN)
5. Membranoproliferative glomerulonephritis (MPGN).

B.Secondary nephrotic syndrome causes include

1. Henoch-scholein purpura
2. SLE
3. Vasculitis
4. Polyarteritis nodosa
5. Wegener's granulomatosis
6. Systemic infections
    - post streptococcal GN
    - Hepatitis B
    - Congenital or secondary syphilis
    - Ventriculo atrial shunt
    - Subacute bacterial endocarditis
    - HIV
    - Malaria

7. Drugs

    - Gold
    - D-penicillamine
    - Captopril

8. Neoplasms

    - Hodgkin's diseas

9. Sickle cell anemia

10. Diabetes

C. Congenital nephrotic syndrome

Sex

In children having onset less than 8 years, the ratio of males to females varies from 2:1 to 3:2 in various studies [(3)]. In older children, adolescents and adults, the male to female prevalence is approximately equal. ISKDC data indicate that 66% of patients with either MCNS or FSGS are male, whereas, for MPGN, 65% are female [(4)].

Age

In children who develop NS while younger than 18 years, approximately 75% are under the age of 6 years with peak incidence between 2-3 years [(5)]. The younger the child at onset (with the exception of the first few months of life), the greater the likelihood

that the lesion is MCN [6]. With onset before age 5 years, the likelihood is >90%; the risk for FSGS and MPGN is 7% and 1%, respectively. Conversely, with onset after age 10, the risk for MCN drops to ~50%, and the risk for FSGS and MPGN is almost 30% and 20%, respectively.

The overall prevalence of NS in childhood is approximately 2-5 cases per 100,000 children. The cumulative prevalence rate is approximately 15.5/100,000 [7].

MCN is the most common form in children, and its prevalence is inversely proportional to the age at onset (i.e. the younger the child, the more likely the histology will show minimal abnormalities on light microscopic evaluation of glomerular histology)[8]. Histologic variations exist within this category in which some patients demonstrate only fusion and smudging of the epithelial cell podocytes while others may demonstrate mild changes within the glomerular mesangium consisting of either proliferation or sclerosis. Since patients with MCN have the highest rate of responsiveness to standard therapy and the best long-term prognosis, the separation of MCN from others is important [9].

IgM mesangial nephropathy (IgM nephropathy) may be a separate entity from MCN. Assignment of type of NS by histologic criteria is based predominately on light microscopic findings. Most patients with isolated IgM mesangial immunofluorescent staining present with clinical characteristics similar to those with MCN. Whether the finding of immune deposits of IgM alters either response to therapy or subsequent

course is controversial.

FSGS is the second commonest type of histologic subtype seen in children and appears to be increasing in frequency [10].

It is not a single disease entity, and attempts to portray it as a uniform entity has led to some confusion in the literature with regard to natural history. FSGS is always a histopathologic diagnosis, and its clinical presentation will vary according to the etiology or cause of the histologic lesion. FSGS may manifest in a fashion that is indistinguishable from MCN, but it may be found only after years of clinical nephrotic syndrome when earlier biopsies have been interpreted as MCN. FSGS is a known consequence of hyper filtration and is regularly seen in patients with reflux nephropathy and in some patients with a single kidney who's other has been lost because of conditions such as multicystic dysplastic kidney disease.

Membranoproliferative glomerulonephritis (MPGN) may manifest as nephrotic syndrome, particularly in older children and adolescents. Its clinical picture is more closely associated with a nephritic picture, but on occasion it may appear similar to MCN or FSGS. Membranous glomerulonephritis (MGN) accounts for less than 1% of the cases of NS in childhood and adolescence and is often associated with hepatitis or other viral disease [11].

Congenital nephrotic syndrome becomes a consideration when nephrosis appears

during the first year of life and particularly in those instances in which the clinical syndrome starts in the first few months.

Pathophysiology

Proteinuria

Heavy proteinuria (albuminuria) is the hallmark of this condition and the primary abnormality in NS. The degree of proteinuria varies considerably from one child to another. Some children will excrete as much as 15 g/m^2/24 hours, and the minimal excretion compatible with the diagnosis is around 1 g/m^2/24 hours (approximately 40 mg/m2 /hour).

The initiating event that produces proteinuria remains unknown. Primary nephrotic syndrome is believed to have an immune pathogenesis, but the precise nature of the process has yet to be defined. It is relation to lymphocyte dysfunction has been suggested, and various studies lend credence to this hypothesis. A highly cationic plasma protein that may neutralize the anionic charge on the glomerular capillary wall has been described in nephrotic children.

Other investigators have noted a decrease in immune responsiveness and related this to alterations in either T-lymphocyte number and/or function[12,13]. The presence of suppresser cytokines or lymphokines has been postulated, and various investigators have shown changes in interleukin-8, interferon-□, IGF-1, TGF-Alfa, and vascular

permeability factor (VPF) [14]. The role of the kinin system is also under investigation, because urinary excretion of kinins is increased during exacerbations of the disease. More recently, alterations in certain molecules expressed in the epithelial cell podocyte, especially nephrin, podocin, and actin, have been shown to have a role in the pathogenesis of the proteinuria [15]. Other researchers have not felt nephrin to be involved in children with MCN. The rate of apoptosis in circulating T-lymphocytes has been found to be increased, and a role for reduced antioxidant defense has been postulated. Despite the regular finding of elevated levels of IgE and an association with atopy in steroid-responsive NS, current data merely suggest a common immune activation rather than a direct association. Leptin is now being investigated for its role in the pathogenesis since, in MCN; serum levels are low at onset of the disease and are associated with elevated serum levels of TGF alfa 1. Additionally, some evidence still exists that genetic factors may be involved in the pathogenesis [16]

In primary nephrotic syndrome, the glomerular capillary permeability to albumin is selectively increased, and this increase in filtered load overcomes the modest ability of the tubules to reabsorb protein. This selective proteinuria (as seen in MCN) is quite different from the more nonselective proteinuria observed in cases of glomerulonephritis. Part of this increase in albumin excretion may be because of the smaller size of the albumin molecule, but since the excretion of some even smaller weight plasma proteins is not proportionally increased, the presence of other factors is obvious. At least 2 hypotheses are proposed to account for this increased permeability.

The traditional hypothesis relates to changes in the anionic composition of the glomerular basement membrane (GBM). In the normal state, the endothelial side of the glomerular capillary wall is negatively charged because of the presence of variety of polyanions along this surface. Thus, the negatively-charged protein, albumin, is less likely to be filtered [17].

In experimental nephrosis and in some children with primary NS, studies have demonstrated a decrease in the normal content of sialic acid (polyanion) from the basement membrane. While such has not been confirmed by all investigators, this deficiency may allow for an increased transport of anionic plasma components.

In such a state, permeability of the glomerular basement membrane would be selectively altered, increasing capillary transport of anionically charged particles such as albumin.

An alternative proposal to explain the heavy proteinuria invokes a primary role for the epithelial cell podocytes. Flattening, retraction, and effacement of the podocyte foot processes are a constant feature of heavy proteinuria.

In the traditional viewpoint, these changes are considered as consequences of the proteinuria. Other investigators believe that primary distortions of the slit diaphragm filaments are present and that there is a redistribution of nephrin from the podocyte slit pores into the cytoplasm.

Hypoalbuminemia is the result of the increased urinary loss of protein. Other factors, however, may contribute to the hypoalbuminemia, among them decreased synthesis, increased catabolism, and increased gastrointestinal losses. Even though most studies have shown that the albumin synthesis rate is not decreased, the capacity to increase hepatic production appears insufficient to compensate for the large urinary losses [18].

Edema

Edema appears to be the natural consequence of the hypoalbuminemia. The classic explanation for edema formation is a decrease in plasma oncotic pressure (as a consequence of low serum albumin) causing an extravasation of plasma water into the interstitial space. The resulting contraction in plasma volume would theoretically lead to a decrease in renal perfusion and hence to stimulation of the renin-angiotensin system [19]. This hormonal effect coupled with an increase in the synthesis and secretion of antidiuretic hormone (related to the decrease in effective plasma volume) would lead to an increase in renal tubular reabsorption of sodium and water. The net result of the combination of Starling forces, reduction in renal perfusion (GFR), and increased hormonal activity would be avid reabsorption of both sodium and water, leading to either maintenance or furthering of the edema [20].

While the above hypothesis on the pathogenesis of edema is attractive, certain experimental data do not completely support this traditional concept. First, the plasma

volume has not always been found to be decreased [21] and, in fact, in most adults, measurements of plasma volume have shown to be increased.

Most (but not all) studies demonstrated a reduced plasma volume only in young children with MCNS. Additionally, most studies have failed to document elevated levels of renin, angiotensin, or aldosterone, even during times of avid sodium retention. Active sodium reabsorption also continues despite actions that should suppress renin effects (i.e., albumin infusion,

ACEI administration). Coupled with these discrepancies is the fact that, in the steroid-responsive nephrotic syndrome, diuresis usually begins before plasma albumin has significantly increased and before plasma oncotic pressure has changed. Some investigators have demonstrated a blunted responsiveness to atrial natriuretic peptide (ANP) despite higher than normal circulating plasma levels of ANP.

Thus, that the precise cause of the edema and its persistence is uncertain should be apparent. A complex interplay of a variety of physiologic factors (i.e., decreased oncotic pressure, increased activity of aldosterone and vasopressin, diminished atrial natriuretic hormone, activities of various cytokines and physical factors within the vasa recti) probably contribute to the accumulation and maintenance of edema.

Hyperlipidemia

Cholesterol levels are usually consistently elevated and an inverse relationship of

cholesterol and albumin is well known [22]. Serum HDL levels are low [23]. 60 % of nephrotics have type IIa or IIb type of hyperlipidemia, 30 % have type V and 10 % or less have type III or IV.

Both increased hepatic synthesis (triggered by hypoalbuminemia) and reduced lipoprotein lipase levels are thought to be the cause for hyperlipidemia. The risk of future atherosclerosis may be increased [22].

Clinical features

Regardless of the type of NS (the histopathologic type), the major clinical manifestation is edema, which is the presenting symptom in about 95% of children. The edema in the early phase is intermittent and insidious; even its very presence may not be appreciated. It usually appears first in areas of low tissue resistance (i.e., periorbital, scrotal, and labial regions) and may progress either rapidly or quite slowly. Ultimately, it becomes generalized and can be massive (anasarca). It is typically dependent in nature, more noticeable in the face in the morning (upon arising) and predominately in lower extremities later in the day. It is pitting in nature. In cases with marked edema, the skin may ooze clear fluid and appear thinner than usual.

An occasional child with NS will present with gross hematuria. The frequency of macrohematuria depends on the histologic subtype of NS. It is more common in those patients with MPGN than in other causes, but its frequency in MCNS has been reported

to be as high as 3-4% of cases. Statistically, a higher percentage of patients with FSGS have microhematuria than those with MCNS, but this is not helpful in differentiating between types of NS in the individual patient. Oliguria is a common occurrence irrespective of the etiology [23].

Regardless of the type of NS, anorexia, irritability, fatigue, abdominal discomfort, and diarrhea are common. If ascites is marked, respiratory distress is not uncommon. An occasional child will present with fever and septic picture; the peritoneum is often the site of the infection. Streptococcus pneumoniae is the most frequent organism responsible for peritonitis in this population, but Staphylococcus aureus and Escherichia coli are commonly recovered. Symptoms of a urinary tract infection are occasionally present.

A history of a respiratory tract infection immediately preceding the first clinical signs of the disease is frequent, but the relevance to causation is uncertain. A history of prior allergic events is common, and atopy has been reported in approximately 40-50% of children with MCN [24].

Treatment

In children with presumed minimal change disease Prednisolone should be administered at a dose of 2 mg/kg/day (60mg/m2/day) in 2 or 3 doses for 4 to 6 weeks then the dose should be tapered to 40mg/m2/day given every other day. Alternate day

dose is tapered and stopped over 2 to 3 months [35].

Cyclophosphamide has been shown to prolong the remission and reduce the number of relapses [36].

Other drugs tried in the treatment of complicated nephrotic syndrome mainly steroid resistant nephrotic syndrome are cyclosporine [37], tacrolimus [38], and mycophenolate.

ACE inhibitors may be used to reduce the proteinuria [39].

Congenital Nephrotic Syndrome

Infants who develop nephrotic syndrome within the first 3 mo of life are considered to have congenital nephrotic syndrome. The most common cause of this syndrome is Finnish type congenital nephrotic syndrome, an autosomal recessive disorder that is most common in populations of Scandinavian descent (1:8,000 incidence).

This type of congenital nephrotic syndrome is caused by a mutation in the *NPHS1* gene located on chromosome 19, which encodes a protein, nephrin. Nephrin is a key component of the slit diaphragm of the glomerular epithelial cell and is thought to play an essential role in the normal function of the glomerular filtration barrier.

The major pathologic features of the Finnish type of this syndrome are dilatation of the proximal tubules, mesangial hypercellularity, and glomerular sclerosis. Infants with the Finnish type of congenital nephrotic syndrome present with massive proteinuria (detectable in utero by increased a-fetoprotein), a large placenta, and marked edema. Additional clinical features include prematurity, respiratory distress, and separation of the cranial sutures. The natural history of the disease is one of persistent edema, recurrent infections, and progressive renal failure with death by the age of 5 yr. Corticosteroids and immunosuppressive agents are of no value.

ACE inhibitors, indomethacin, and unilateral nephrectomy may diminish proteinuria and ameliorate the nephrotic state. However, the preferred treatment includes bilateral nephrectomy, chronic dialysis, aggressive nutritional support, and eventual kidney transplantation. In families at risk for the Finnish type of congenital nephrotic syndrome, antenatal diagnosis is suggested by an elevated amniotic fluid a-fetoprotein level and the diagnosis may be confirmed by DNA analysis.

Other causes of congenital nephrotic syndrome include congenital infections such as syphilis, toxoplasmosis, rubella, and cytomegalovirus. HIV and hepatitis B have also been reported to cause nephrotic syndrome in the neonatal period. The nephrotic state, which is generally less severe than the Finnish type of congenital nephrotic syndrome, may improve or resolve with treatment of the underlying infection.

Diffuse mesangial sclerosis is a rare glomerular disease seen in a minority of children with congenital nephrotic syndrome. The characteristic pathologic finding is progressive sclerosis of the glomerular mesangium, and the clinical picture is one of rapid loss of renal function, with end-stage renal disease developing within months to years. Diffuse mesangial sclerosis may occur as an isolated disease or as part of *Denys-Drash syndrome,* a condition also characterized by Wilms tumor and male pseudohermaphroditism, caused by a mutation in the Wilms tumor gene *(WT1)* on chromosome 11.

REVIEW OF LITERATURE

The incidence of nephrotic syndrome in western countries varies between 2 to 2.5/100,000children per year [25]

Srivasthava reported a incidence of 1.4% among pediatric patients in India[7]

Age and sex

Prospective studies by ISKDC from 1967-1976 reported that 60% of children with MCD were 2-6 years of age [9].

White (1970) [26] reported a male to female ratio 2:1 which diminishes with advancing age.

Srivasthava et al [27] studied 206 cases and found that male to female ratio was 3:1 in younger children and 7:1 in older children. But generally children with SRNS have higher age of onset {8.2 years [26]}

In 80 to 85 % of children, nephrotic syndrome results from MCD which is generally steroid responsive [28]. But this high incidence of primary nephrotic syndrome due to MCD may not be applicable to all societies.

For example most nephrotic African children have obvious structural glomerular lesions and are unresponsive to steroids [29].

All the studies done in nephrotic syndrome so far showed, that the children with nephrotic syndrome other than MCN poorly respond to steroids. Even in MCN about 10% of cases are resistant [30]

Alexandru R. Constantinescu, Hetal B. Shah, Edward F. Foote, and Lynne S. Weiss et al [31] From the Department of Pediatrics, Division of Pediatric Nephrology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, New Jersey; They did a prospective study to identify factors at initial presentation that could predict the relapse and resistant pattern in the first year after diagnosis, without taking into consideration the histopathology found on renal biopsy. Variables selected in the study were age, sex, race, presence or absence of hematuria, and days to remission.

Results: Of 70 patients, 14 were excluded because of insufficient data. There were 38 males (67.9%) and 18 females (32.1%), giving a male: female ratio of 1.8:1. Median age at presentation was 3.25 years (range: 1.5-13). Of all the patients, 23 were infrequent relapse (41.1%), 9 were frequent relapse (16.1%), and 24 were Steroid dependant (42.9%). Hematuria was present initially in 26 patients (46.4%), and absent in 30 (53.6%). Age, sex, race, and hematuria, as independent variables, were not predictors of relapses in the first year. However, using a stratified analysis based on the presence or absence of hematuria, we found that if the remission occurred within the first week of therapy, the patients without hematuria were more likely to be infrequent relaps. The

sensitivity and specificity of this finding was 67% and 89%, respectively, with a positive predictive value of 94%.

Conclusion of this study was the rapidity of initial response to steroid therapy combined with the presence of hematuria could predict future relapses and should be well documented.

Hideo shiiki and Kazuhiro et al [(40)] from Nara medical university did a study on clinical course, predictors of renal outcome in patients with steroid resistant nephrotic syndrome when clinical and histopathological features at presentation have been evaluated by multivariate analysis, serum creatinine concentrations (>1.5 mg/dl) and the presence of tubulo-interstitial lesions (>20%) are significant positive predictors of progression to ESRD.

Histopathological distribution of nephritic syndrome

Type	White et al[26]	Habib et al.,[41]	ISKDC [4]	ICH & HC [44]
MCD	76.5%	51.5%	76.4%	62.2 %
FSGN	8.2%	11.5%	6.9%	12.4 %
MGN	1.3%	8%	6.9%	2.1 %
MPGN	6.2%	13%	7.5%	7.8 %
OTHERS	5%	13.5%	7.7%	6.0 %
DMP	2.8%	1%	0%	15.9 %

Vipul c.chitalia et al.,[42] did a retrospective study to predict the renal survival in focal glomerulosclerosis on 111 patients who were diagnosed at Christchurch hospital from 1965 to 1998. the predictors of outcome included age, gender, blood pressure, serum albumin, plasma creatinine, presence of hematuria and amount of proteinuria (at the time of presentation).An injury score (combination of % of sclerosed glomeruli and proportion of tubulointerstitial fibrosis) was derived from a review of the initial renal biopsy. The median renal survival was 16.4 years. The best single variable model was that using the proportion of tubulointerstitial fibrosis. However, inclusion of plasma creatinine significantly improved the fit of the model.

South west pediatric nephrology study group [34] prospectively monitored 75 pediatric patients with FSGS most of whom were treated with steroids after a mean

follow-up of 57 months. 21% of these patients developed ESRD among which 23% had decreased GFR and 33% had persistent proteinuria. Only 11% went into remission.

Nesrin Besbas, Rezan Topaloglu, Omit Saatci, Aysin Bakkaloglu et al [43] from the Department of Pediatric Nephrology, Hacettepe University School of Medicine, Ankara, Turkey did a prospective long term follow up study of children with steroid resistant nephrotic syndrome. Out of 215 children with steroid-resistant primary NS, 164 had been followed from 1 to 10 years. The children had a mean age of 8.2 years, with a range from 1 to 16 years. Steroid resistance was more common in children over six years of age compared with the other age groups. Hematuria was seen in 68 of the 164 children (41%); hypertension in 41 (25%); and hyperlipidemia in 112 (68%). Hypocomplementemia was noted in 24 of the 65 (37%) children in whom complement concentrations were determined. Renal biopsy was performed in 117 of the children.

Pathologic changes consisted of minimal change nephrotic syndrome (MCNS) in 14 children (12%), membranoproliferative glomerulonephritis (MPGN) in 45 (38%), focal segmental glomerulosclerosis (FSGS) in 20 (25%), mesangial proliferation (MP) in 23 (20%), and membranous glomerulonephritis in 6 children (5%). Cyclophosphamide (2 mg/kg/day) was given to 164 patients, with complete remission and partial remission rates of 20.7% (34 of 164 children) and 24.4% (40 of 164 children), respectively.

In this group, sustained remission and sustained partial remission rates were found

in 20% (32 children) and 13% (21 children), respectively. Chlorambucil was given to 40 children with steroid- and cyclophosphamide-resistant nephrotic syndrome, with total remission and partial remission rates of 20% (eight children), and 12.5% (five children), respectively. These rates did not change during the follow-up. Thus, cyclophosphamide is valuable in the treatment of children with steroid-resistant NS with a variety of histologic changes [32]

Treatment

The ISKDC reports that 91.8% of patients who responded had minimal-change glomerulonephritis, compared with only 25% of patients who did not respond.

In patients who do not respond to corticosteroid treatment and are younger than 6 years, approximately 50% had minimal-change glomerulonephritis; in those older than 6 years, only 3.6% had minimal-change glomerulonephritis.

The Southwest Pediatric Nephrology Study Group reports that 63% of patients with diffuse membranous hypercellularity and approximately 30% of patients with focal glomerular sclerosis responded to corticosteroid therapy. Congenital nephrotic syndrome is usually resistant to corticosteroid treatment.[34]

STUDY JUSTIFICATION

Steroid Resistant-Nephrotic Syndrome (SRNS) is a chronic, progressive disorder a relatively uncommon renal disease in children, affecting up to 20% of all children with NS. Of these, most will have FSGS on biopsy, with smaller number having MCN. It causes morbidity and mortality due to persistent edema, hypertension, hyperlipidemia, thrombosis and infection. Progression to renal failure was thought to be inevitable in survivors. Recent insights into the pathogenesis of the disease have identified several responsible genes and proteins. Although numerous treatment regimens have been used, approximately 70% of FSGS are unresponsive to steroids, and no other regimen has been found that adequately treats SRNS and FSGS. Because of the lack of effective treatment and the poor prognosis with progression to chronic renal insufficiency and end stage renal disease, SRNS represents a significant therapeutic dilemma for pediatric nephrologists. Hence this study has been concentrated mainly on children with SRNS but at the same time, those children with steroid responsive nephrotic syndrome have been studied since this group constitutes the major type.

AIM OF THE STUDY

1. To find out the incidence, age and sex distribution and also the distribution of various types of steroid resistant nephrotic syndrome in a given period.

2. Predictors of the response to steroids from clinical, biochemical and radiological characteristics of children presenting with features of nephrotic syndrome.

3. To find out the histopathological type in steroid resistant cases.

SUBJECTS AND METHODS

Study design

Prospective case study, conducted in the Department of nephrology, Institute of child health, Chennai.

Study period:

Jan 2006 to Sep 2007

Study place

Institute of child health and Hospital for children

Study population

Children attending the nephrology outpatient department who fulfill the criteria of nephrotic syndrome and who had undergone treatment for at least 6 weeks of steroid were included.

Diagnostic criteria for nephrotic syndrome

ISKDC defines nephrotic syndrome as

1. Massive proteinuria of 40mg/m^2/hr,

2. Hypoalbuminemia <2.5 gm/dl
3. Hypercholesterolemia >220mg /dl and with or without edema.
4. Spot protein creatinine ratio >3.5
5. 24 hour urine protein >50mg/kg/day

Maneuver

Clinical data

Complete history was obtained from parents regarding the illness from onset. A detailed clinical examination and laboratory evaluation was done in all patients with special emphasis on unusual features like high Blood Pressure, hematuria, and infections.

Follow up

All newly diagnosed NS cases were followed every 2 weeks. They were treated with steroids as per APN regime. The parents were given clear instructions on diet and as to how to take the drugs.

Response to steroids was monitored with urine albumin and more than 2+ was taken as significant proteinuria.

All these children were followed-up for 8 weeks. After 8 weeks Steroid responders were released from follow-up. Those children with SRNS underwent renal biopsy and histopathology and were entered.

Investigations

Urine

Heat coagulation method was done to detect proteinuria. Urine microscopy and culture was sent at initial presentation and also during subsequent relapses.

Spot urine protein/creatinine and 24 hours urine protein estimation was done for all the patients who got admitted during the first or subsequent visits and only spot urine protein/creatinine was estimated for the patients who came with a relapse and got treated as outpatients.

Blood biochemistry

Blood urea, serum creatinine, serum electrolytes (Na, K, Ca and Po4) serum protein (total, albumin, globulin) and serum cholesterol was estimated for the children who got admitted for the first visit and also during the subsequent visits when necessary.

ANA titer was done whenever necessary. Australia antigen was tested for all the patients whenever the kit was available.

Routine hemogram, Mantoux test and x-ray chest were done for all the patients before starting them on steroids. For patients taking cyclophospamide weekly hemogram was done.

Ultrasound

Ultra sonogram of the kidney was done for all the patients during first admission and they were classified into 4 groups

1. Normal study- Renal cortical echo texture is less than that of liver parenchymal echo texture.

2. Grade I RPD (renal parenchymal disease)- renal cortical echo texture is equal to that of liver parenchymal echo texture.

3. Grade II RPD- renal cortical echo texture is more than that of liver parenchymal echo texture.

4. Grade III RPD-renal parenchymal (cortex and medulla) echo texture is more than that of liver parenchymal echo texture, with loss of corticomedullary differentiation.

Renal biopsy

Written consent was obtained from the parents before biopsy. Biopsy was done under local anesthesia with a trucut needle or biopsy gun under strict aseptic precautions after localizing the site of the renal biopsy by ultrasound and after ruling out coagulation abnormalities.

The indications for doing a renal biopsy were:

1. Atypical presentation- gross hematuria, persistent hypertension or renal failure.
2. Steroid resistant nephrotic syndrome.
3. Steroid dependent nephrotic syndrome.
4. Frequent relapses.
5. Before starting cytotoxic drug therapy.
6. Systemic manifestations.

But biopsy could not be done for all the patients mentioned above because of various reasons due to unwillingness of the parents for biopsy or presence of infection at that time, etc.

Treatment

All the patients with the first episode of nephrotic syndrome were admitted for investigations and treatment. Patients with a focus of sepsis were treated with appropriate antibiotics prior to steroid therapy. And subsequently were started on the following regime.

Prednisolone 2 mg/kg/day (maximum 60mg) in divided doses was given for 4 weeks. After that the same dose was given as a single dose on alternate days for 4 weeks. Then the dose was tapered by 10mg every 2 weeks.

The course after the therapy was defined as follows:

1) Remission

A reduction in the urinary protein excretion to trace or nil by heat coagulation method for three consecutive days.

2) Relapse

Appearance of proteinuria (2+ or more) for three consecutive days after a period of response for at least 4 weeks.

Frequent relapse

Any responder who had two or more relapses within 6 months of the initial response or three relapses within one year period

3) Steroid depender

Patients who need prolonged and continued maintenance of steroid therapy. Discontinuation or reduction in the dose results in relapse of proteinuria within 2 weeks.

4) Steroid resistant

Persistent proteinuria (2+ or more) after 8 weeks of daily divided doses of steroid.

Treatment of Steroid resistant cases

If there is persistent proteinuria even after 4 weeks of daily steroids (2 mg/kg/day), then the same dose is continued daily for another 4 weeks. If there is no remission even after 8 weeks of full dose of steroids, it is labeled as steroid resistant nephrotic syndrome. In these cases, the dose of Prednisolone is tapered to 0.5mg/kg and continued. Simultaneously i.v cyclophospamide was added in a dose of 500 mg/m^2 and 6 monthly pulses were given.

Other drugs that we are using in our hospital include Methyl Prednisolone, ACE inhibitors like Enalapril to reduce the proteinuria and newer drugs like Tacrolimus.

Hypertension was treated with alpha methyl dopa in the dose of 10 to 20 mg/kg/day in divided doses. Renal failure was treated conservatively and if failed then peritoneal dialysis was done.

Diet

All patients were given a normal protein diet of high biological value with no added salt. In the presence of severe edema salt restricted diet was given in addition to furosemide or spiranolactone or fresh frozen plasma.

OBSERVATION AND DISCUSSION

Types of nephrotic syndrome

Table 1

Type	N	%
Steroid responsive nephrotic syndrome	112	60.5
Infrequent relapse	21	11.4
Frequent relapse	6	3.2
Steroid dependant nephrotic syndrome	24	13.0
Steroid resistant nephrotic syndrome.	22	11.9
Total no of patients	185	100

In our hospital, nephrotic syndrome constitutes 9% of the patients attending the nephrology out patient department. This is the second commonest renal problem in children, the first being acute nephritic syndrome. The incidence of steroid resistant nephrotic syndrome in our study was 11.9% (22). Steroid responsive nephrotic syndrome is the predominant type with 60.5% (112), steroid dependant type follows with 13% (24) and significant number of children developed relapses 14.6% (27).

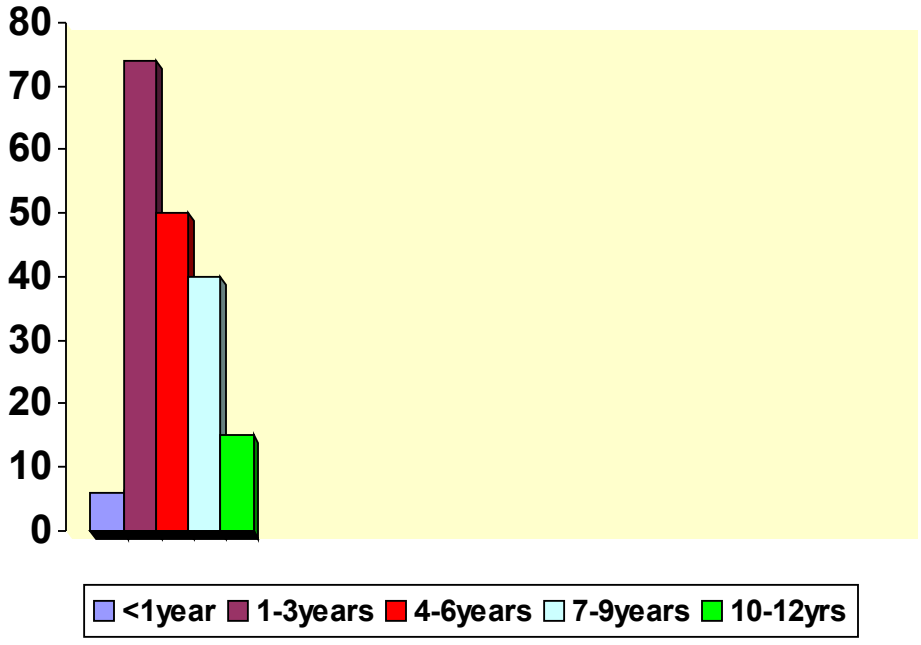
Among the relapse group 11.4% (21) children had infrequent relapse. In our study

2 children had secondary nephrotic syndrome, one due to Hodgkin's lymphoma who responded well to steroids now under follow up in hematology department and another had Systemic lupus erythematosis who was lost to follow up.

Nephrotic syndrome age distribution

Table 2

Age	n	%
<1 year	6	3.2
1 – 3 years	74	40.0
4 – 6 years	50	27.0
7 – 9 years	40	21.6
10 – 12 years	15	8.1



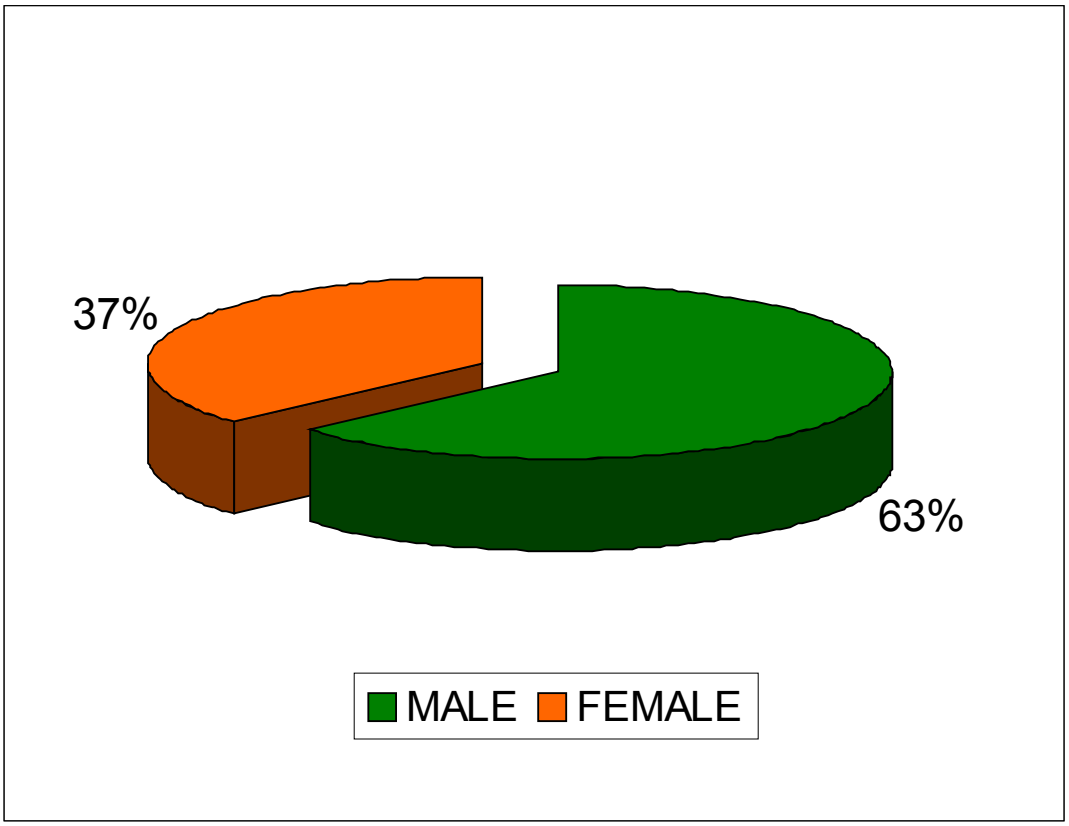
Steroid resistant & responsive Nephrotic syndrome age distribution

Table 3

Age	Steroid resistant (n=22)		Steroid responsive(n=112)	
	Number	**%**	**Number**	**%**
<1 year	1	4.5	3	2.5
1 – 3 years	8	36	42	37.5
4 – 6 years	7	32	32	28.5
7 – 9 years	5	23	23	20.5
10-12years	1	4.5	12	11

In this study, mean age at presentation was 4 years for both steroid responsive and resistant group. The range is from birth to 12 years. Older age of onset for SRNS has been noticed in various studies however has not been found in our study. In our study no congenital nephrotic syndrome was reported.

Sex distribution of nephrotic syndrome



Sex distribution Steroid resistant & responsive nephrotic syndrome

Table 4

Sex	Steroid resistant (n=22)		Steroid responsive (n=112)	
	Cases	%	Cases	%
Male	14	64	73	65
Female	8	36	39	35

Sex distribution

The overall male to female ratio in this study is 2:1. Both the groups show male predominance. Male predominance was reported in a number of other studies too.

White (1970) reported a male to female ratio 2:1 which diminishes with advancing age.

Srivasthava et al reported a male to female ratio of 3:1 in younger children and 7:1 in older children.

Family history

Family history of nephrotic syndrome was noticed in 2 patients with resistant group (both DMP) and in 3 patients with responsive group.

This is in contrast to the study by Habib et al., in which FSGS is the common lesion in familial nephrotic syndrome.

Atypical features in steroid resistant and steroid responsive nephrotic syndrome

Table 5

S.NO.	Features	Resistant (n=22)		Responsive (n=112)	
		No of cases	%	No of cases	%
1.	Hematuria	7	32%	2	1.7
2.	Hypertension Transient Persistent	6 5 1	27%	Nil	0
3.	Renal failure Transient Persistent	10 9 1	45%	16 16	14

Atypical manifestations like hematuria, hypertension and renal failure are found to be more common in steroid resistant nephrotic syndrome. The reason behind this may be that SRNS produces structural damage to the kidney unlike MCN where the basic pathology is only the loss of negatively charged glycosaminoglycons. 32% (7) of children with steroid resistance had hematuria as compared to 1.7 % (2) in responsive group.

Hypertension at the onset was documented in 27% (6) of children with resistance. These children were treated with short term methyl dopa; out of this one child had persistent hypertension and progressed to chronic renal failure.

Elevated renal parameter was noticed in 45% (10) of patients with resistance and 14% (16) of patients in response group. One child required peritoneal dialysis. Child who developed chronic renal failure is under follow up. These factors predict the development of resistance with statistical significance ($p<0.05$).

Serum albumin level in steroid resistant and responsive nephrotic syndrome

Table 6

Serum level (g/dl)	Steroid responsive (n=112)		Steroid resistant (n=22)	
	Number	%	Number	%
>2.5	11	10	2	9
1.5 – 2.5	101	90	19	86
<1.5	Nil	0	1	5

Serum cholesterol level in both groups

Table 7

Serum level(mg/dl)	Steroid resistant (No = 22)		Steroid responsive (No =112)	
	No of cases	%	No of cases	%
200-300	1	5	13	12
300-400	8	36	57	51
400-500	8	36	25	22
500-600	4	18	12	11
>600	1	5	5	4

Serum albumin is found to be low in (<2.5gm/dl) in both groups. Even though serum cholesterol is found to be high with resistant group than in responsive group, statistically the difference is insignificant (p>0.05). In children with steroid resistant nephrotic syndrome hypoalbuminemia was found in 91 % (20) of patient as against 90 % (101) of patients with responsive group and serum cholesterol >400mg/dl was found in 59 % of patients with SRNS as against 37 % in response group.

Thus the value of serum albumin and serum cholesterol levels will not give a clue to the type of nephrotic syndrome.

Type of infections in both groups

Table 8

Infection	Steroid resistant (n=22)		Steroid responsive (n=112)	
	No. of cases	%	No. of cases	%
UTI	6	27	13	11
LRI	2	9	2	2
Peritonitis	1	4.5	-	-
Tuberculosis	-	-	2	2

Urinary tract infection is more common in steroid resistant nephrotic syndrome 27% (6) than responsive group 11% (13).

The reason for this difference is not known. One child developed peritonitis in resistant group and two children in responsive group showed positive mantoux. RGJ

(resting gastric juice) for AFB (acid fast bacilli) was done in positive mantoux cases which were negative. They received anti tuberculosis treatment according to IAP recommendation. 4 children had respiratory infection, two in each group.

Ultrasound findings in both groups

Table 9

USG	Steroid resistant (n=22)		Steroid responsive(n=112)	
	No. of cases	%	No. of cases	%
Normal	7	32	84	75
Grade 1 RPD	7	32	26	23
Grade 2 RPD	8	36	2	2

Abnormal ultrasound findings in the form of grade 1 or grade 2 RPD was seen in 68% (15) of children with steroid resistant nephrotic syndrome as against 25% (28) in responsive group ($p<0.05$).

X ray findings in both groups

Table 10

CXR	Steroid Resistant (n=22)		Steroid Responsive(n=112)	
	No. of cases	%	No. of cases	%
Normal	14	63.6	97	87
Pleural effusion	8	36.4	15	13

Pleural effusion was found in 36.4 % (8) of children with resistant nephrotic syndrome and was 13 % (15) in responsive group ($p<0.05$). From this it can be concluded that in the presence of abnormal radiological features in a child with nephrotic syndrome then, the probability of having a SRNS is high.

Table 11

	Steroid responsive nephrotic syndrome		Steroid resistant Nephrotic syndrome		*p-value*[+]
	No. of cases	**%**	**No. of cases**	**%**	
Age					
<1 year	3	50	1	25.0	
1 – 3 years	42	57	8	16.0	
4 – 6 years	32	64	7	17.9	
7 – 9 years	23	57	5	17.9	0.90
10 – 12 years	12	80	1	7.7	
Sex					
Male	39	83.0	8	17.0	1.00
Female	73	83.9	14	16.1	
Hematuria					
Absent	110	88.0	15	12.0	0.00
Present	2	22.2	7	77.8	
Hypertension					
Absent	112	87.5	16	12.5	0.00
Present	-	-	6	100.0	
UTI					
Absent	99	86.1	16	13.9	0.09
Present	13	68.4	6	31.6	
Urea					
<40 mg/dl	96	89.7	11	10.3	0.00[*]
41 - 100mg/dl 101-200mg/dl	16	61.5	10	38.5	

	-	-	1	100.0	
Creatinine					
<1mg/dl	106	89.8	12	10.2	0.00
1-2 mg/dl	6	37.5	10	62.5	
Albumin					
>2.5 gm/dl	11	84.6	2	15.4	
1.5 –2.5 gm/dl	101	84.2	19	15.8	0.08
<1.5 gm/dl	-	-	1	100.0	
Cholesterol					
200-300mg/dl	13	92.9	1	7.1	
300-400mg/dl	57	87.7	8	12.3	
400-500mg/dl	25	75.8	8	24.2	
500-600mg/dl	12	75.0	4	25.0	0.41
>600mg/dl	5	83.3	1	16.7	
Mantoux					
Negative	110	83.3	22	16.7	1.00
Positive	2	11.2	-	-	
USG					
Normal	84	92.3	7	7.7	
Grade I RPD	26	78.8	7	21.2	0.00*
Grade II RPD	2	20.0	8	80.0	
Chest x-ray					
Normal	97	87.4	14	12.6	0.03
Pleuraleffusion	15	65.2	8	34.8	

*Chi-square for trend

+Chi-square

Univariate Logistic Regression

	O.R.	95% C.I.	p-value
Hematuria			
Absent	1.0	Reference	0.00
Present	25.7	4.9 , 135.2	
Urea			
<40 mg/dl	1.0	Reference	0.00
>40 mg/dl	6.0	2.2 , 16.1	
Creatinine			
<1mg/dl	1.0	Reference	0.00
1-2 mg/dl	14.7	4.5 , 47.7	
USG			
Normal	1.0	Reference	0.00
RPD	6.4	2.4 , 17.4	
Chest x-ray			
Normal	1.0	Reference	0.01
Pleural effusion	3.7	1.3 , 10.3	

Hematuria was observed in 7 out of 22 children with steroid resistant nephrotic syndrome when compared to 2 out of 112 among steroid sensitive nephrotic syndrome. Odds ratio of having hematuria is 25.7 among steroid resistant nephrotic children when compared to steroid responsive children {OR 95% CI (25.7 &4.9, 135.2)}

Serum urea level was >40mg/dl in 11/22 children with steroid resistant nephrotic syndrome when compared to 16/112 among steroid sensitive nephrotic syndrome. Odds ratio of having serum urea level >40mg/dl is 6 among steroid resistant nephrotic children when compared to steroid responsive children {OR 95% CI (6&2.2, 16.1)}

Serum creatinine level was more than 1 mg/dl in 12 out of 22 children in steroid resistant group when compared to 6 out of 112 among steroid sensitive group.

Odds ratio of having serum creatinine level >1mg/dl is 14 {OR 95% CI (14.7&4.5, 47.7)}.

USG shows RPD in 15 out of 22 children with steroid resistant nephrotic syndrome and 28 out of 112 among steroid sensitive nephrotic syndrome. Odds ratio of children showing RPD in USG is 6.4 {OR 95% CI (6.4&2.4, 17.4)}

Chest X ray showed pleural effusion in 8 out of 22 children with steroid resistant nephrotic syndrome and 15 out of 112 among steroid sensitive nephrotic syndrome. Odds ratio of children showing pleural effusion in chest X ray is 3.7 {OR 95% CI (3.7&1.3, 10.3)}

Multiple Logistic Regression

	O.R.	95% C.I.	p-value
Urea			
<40 mg/dl	1.0	Reference	0.004
>40 mg/dl	4.7	1.6 , 13.5	
USG			
Normal	1.0	Reference	0.002
RPD	5.3	1.9 , 14.9	

Histopathological findings in steroid resistant nephrotic syndrome

S.NO.	Type	Steroid resistant	
		No .of cases	%
1.	MCN	**3**	**14**
2.	FSGS	**6**	**27**
3.	DMP	**8**	**36**
4.	MPGN	**-**	**-**
5.	Membranous	**1**	**5**
6.	Biopsy not done	**4**	**18**

Histopathological type

DMP is the commonest type other than MCN found in this study. It constitutes 9% of the patients in contrast to other studies, where it constitutes only 2.3 to 5.3%. It forms 36% of all patients with steroid resistant nephrotic syndrome followed by FSGS that comprises 27% of patients with resistant nephrotic syndrome and 14% MCN type.

Even though the distribution of various types differs slightly with other studies the more striking difference in this study is the occurrence of a significant proportion of cases with DMP

SUMMARY

1. Nephrotic syndrome is the second commonest renal problem in children preceded by acute nephritic syndrome. It constitutes 9% of patients attending the nephrology outpatient.

2. The higher age of onset for SRNS than responsive nephrotic syndrome noted in other studies was not noticed in this study. Males are affected more than females in both groups (2:1).

3. Atypical manifestations like hematuria, hypertension and renal failure are more common in resistant nephrotic syndrome and they predict development of resistance as well.

4. The incidence of hypoalbuminemia and hypercholesterolemia are same in both groups and these are poor predictors of the type.

5. Radiology plays a significant role in predicting steroid resistance. USG of kidney is abnormal in significantly more number of patients with SRNS. Pleural effusion is more common in resistant group than responsive group

6. Urinary tract infection is more common in steroid resistant nephrotic syndrome.

7. DMP is the commonest non minimal lesion and it is the commonest histopathological type in SRNS followed by FSGS and MCN.

CONCLUSION

Presence of hematuria, hypertension, and elevated renal parameters in the initial presentation with abnormal ultrasound findings predicts the future development of steroid resistance. Hence these children should be monitored periodically.

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